PATENT
Attorney Docket 4205.2US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: ________________

Date of Deposit with USPS: ______________________

Person making Deposit: _________________________________

APPLICATION FOR LETTERS PATENT

for

DENDRITIC CELLS ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T-CELL RESPONSES

Inventors:

Delphine Gabrielle Josette Rea
Cornelis Johannes Maria Melief
Rienk Offringa

Attorney:
Allen C. Turner
Registration No. 33,041
TRASKBRITT
P.O. Box 2550
Salt Lake City, Utah 84110
(801) 532-1922

TITLE OF THE INVENTION

DENDRITIC CELLS ACTIVATED IN THE PRESENCE OF GLUCOCORTICOID HORMONES ARE CAPABLE OF SUPPRESSING ANTIGEN-SPECIFIC T-CELL RESPONSES

[0001] Reference to Related Application. This application claims priority from Provisional Application Serial No. 60/157,442, filed October 4, 1999.

[0002] Technical Field. The invention relates to the field of medicine. More in particular, the invention relates to the field of immunotherapy.

BACKGROUND OF THE INVENTION

[0003] The remarkable immunostimulatory properties of dendritic cells ("DC") reside in their ability to transport antigens from peripheral tissues to lymphoid organs where they present these antigens to T-cells in an optimal costimulatory context (1). To achieve this complex sequence of events, DC exist in different functional stages. Immature DC behave as sentinels in peripheral tissues where they efficiently capture antigens. Upon pathogen invasion, induction of protective T-cell responses require the activation of immature DC into mature immunostimulatory cells. DC activation is triggered in inflamed tissues by cytokines such as IL-1 and TNF-a and by bacterial components such as lipopolysaccharide (LPS) (2, 3). Activated DC migrate to T-cell areas in the lymph nodes while upregulating their costimulatory capacities and optimizing their antigen presenting functions. Upon interaction with antigen-specific T-cells, DC activation is further completed through engagement of the receptor-ligand (1) pair CD40-CD40L, leading to the production of IL-12 (4, 5, 6), a key cytokine for T helper (Th) type 1 and cytotoxic T lymphocyte (CTL) priming (7).

[0004] Antigen Presenting Cell (APC) activation through CD40-CD40L interactions represents an important immunoregulatory step for the establishment of protective T-cell immunity against pathogens and tumors (8, 9, 10). This process also plays a key role in the onset of destructive T-cell-mediated disorders such as autoimmune diseases, allograft rejection and graft versus host disease (11, 12, 13). The current treatment of these disorders largely relies on the administration of glucocorticoids (the abbreviation "GC" is used herein for the terms "glucocorticoids" and "glucocorticoid"), which exert potent anti-inflammatory and immunosuppressive effects. Because GC negatively interfere with many aspects of T-cell

activation, such as IL-2-driven proliferation and inflammatory cytokine production (reviewed in 14), activated T-cells have long been considered as the main targets for GC action. Several lines of evidence now suggest a role for DC in GC-induced immune suppression. Moser et al. (15) found that GC prevented the spontaneous activation of murine DC thereby decreasing their T-cell stimulatory potential. Kitajima et al. (16) showed that GC could hamper the T-cell-mediated activation of a murine DC line. Viera et al. reported that human DC exposed to GC were poor producers of IL-12 upon LPS stimulation (17). These findings only concern loss of typical DC features and, therefore, favor a simple inhibitory role of GC on DC activation. A more complex immunoregulatory action on the DC system has not been considered.

[0005] The present invention resulted from a detailed analysis of the impact of GC on the CD40-mediated activation of monocyte-derived DC. These DC develop after culture with GM-CSF and IL-4 (2, 18) or after transmigration through endothelial cells (19) and are known to mature into the most potent human Thl-type-inducing APC upon CD40 ligation (5, 20). Moreover, these APC can easily be generated in large numbers and are thereby the cells of choice for DC-based modulation of T-cell immunity (21, 22). In contrast to previous studies, the present invention shows that GC, such as dexamethasone (DEX), do not merely prohibit DC activation but converts CD40 ligation on human monocyte-derived DC and is transformed into an alternative activation pathway. DEX profoundly affects the CD40-dependent maturation of human monocyte-derived DC, not only by preventing the upregulation of costimulatory adhesion and MHC surface molecules, but also by causing these cells to secrete the anti-inflammatory mediator IL-10 instead of the Thl stimulatory cytokine IL-12. In agreement with these phenotypic and functional changes, DC triggered through CD40 in the presence of DEX are poor stimulators of Thl-type responses. Most importantly, the present invention shows that such DC are able to induce a state of hyporesponsiveness in Th1 cells, indicating that these cells are capable of active suppression of Th1-type immunity.

SUMMARY OF THE INVENTION

[0006] As mentioned above, the impact of GC on DC has been the subject of several previous studies by others. However, in contrast with the present invention, these studies only highlighted inhibitory effects of GC on the DC system. DEX was found to block the upregulation of CD80, CD86 and MHC class II molecules upon activation of murine spleen DC (15, 16),

whereas very recently DEX was demonstrated to also prevent the differentiation of DC from monocyte precursors (28). In these studies, the inability of DC to acquire high expression of costimulatory and MHC molecules was accompanied with a decrease in their T-cell stimulatory potential, but the effect of GC on IL-12 production was not investigated. On the other hand, Viera et al. found that the effect of GC on LPS-induced DC activation consisted in a 4-fold reduction of IL-12p70 synthesis (17). This partial effect on IL-12 secretion contrasts with the complete suppression of IL-12p70 production which is the subject of the present invention and can be explained by the fact that their GC-treated immature DC were extensively washed prior to LPS stimulation. We indeed found that upon removal of GC, the effects of these drugs on immature DC were rapidly reversible. The continuous presence of GC during CD40 triggering of DC was clearly preferred in order to stably and completely modulate DC activation (data not shown). Taken together, previous findings indicated that the impact of GC on the DC system should be merely interpreted as an inhibitory event. Importantly, the present invention clearly demonstrates that GC, such as DEX, do not simply suppress DC activation but rather redirect this process towards a distinct functional program.

[0007] DC activation through engagement of CD40-CD40L is a key stimulatory event for the generation of effective Th1 and CD4-dependent CTL responses in vivo (10, 36, 37, 38). This pathway, however, is also involved in the development of unwanted T-cell responses leading to autoimmune disease or organ-transplant rejection (11, 12, 13). Until now, treatment of patients suffering from such disorders largely relied on the systemic administration of GC hormones. This treatment does not only suppress pathogenic T-cell responses, but also induces a general state of immunosuppression and metabolic and endocrine side effects. The present invention demonstrates that activation of human monocyte-derived DC through CD40, in the presence of GC such as DEX, results in an IL-10-producing APC that is a poor stimulator for Th1-type responses and that can even confer hyporesponsiveness to Th1 cells. The present invention, therefore, indicates that such DC loaded with appropriate antigens can be exploited as a novel approach for specifically downregulating unwanted T-cell responses in vivo.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Fig. 1. Pretreatment with DEX inhibits the phenotypic changes induced by CD40 ligation.

[0009] Seven days immature DC were cultured for 24h in the absence or the presence of 10^{-6}M DEX and activated via CD40 with the CD8-CD40L fusion protein for 48h. The comparison with immature DC maintained in medium alone is shown. Empty histograms show the background staining with isotype controls MoAb and solid histograms represent specific staining of the indicated cell surface markers. Specific mean fluorescence intensities are indicated. Mean fluorescence intensities of isotype controls were between 3 and 4. Data are representative of 4 independent experiments.

[0010] Fig. 2. DC triggered through CD40 maintain an activated phenotype upon a subsequent DEX exposure. Immature DC were activated with the CD8-CD40L fusion protein. DEX (10^{-6}M) or medium control were added 48h later and cells were analyzed after 2 additional days of culture. The comparison with immature DC maintained in medium alone is shown. Empty histograms show the background staining with isotype controls MoAb and solid histograms represent specific staining of the indicted cell surface markers. Specific mean fluorescence intensities are indicated. Mean fluorescence intensities of isotype controls were between 3 and 5. Data are representative of 2 independent experiments.

[0011] Fig. 3. Pretreatment with DEX does not affect the regulation of DC antigen uptake machinery. Immature DC were incubated in the absence or the presence of 10^{-6}M DEX for 24h and further activated or not via CD40 with the CD8-CD40L fusion protein for 48h. Cells were pulsed for 1h with medium containing either 1mg/ml FITC-BSA or 1mg/ml FITC-mannosylated BSA. Empty histograms show the background autofluorescence, Grey-filled histograms show the background uptake at 0°C. and black-filled histograms show the specific uptake at 37°C. Data are representative of 3 independent experiments.

[0012] Fig. 4. Pretreatment with DEX alters the cytokine secretion profile of CD40-triggered DC.

[0013] DEX-exposed or control immature DC were left in culture without further treatment or stimulated with the CD8-CD40L fusion protein. Culture supernatants were harvested 48h later and IL-10, IL-12p40 and IL-12p70 secretion were analyzed by specific ELISA. Data are representative from 6 independent experiments.

[0014] Fig. 5. Pretreatment with DEX impairs the T-cell stimulatory capacities of DC activated via CD40 and leads to a state of hyporesponsiveness of Th1 cells.

[0015] Allogeneic MLR: nonadherent allogeneic PBMC were cultured with different numbers of CD40-triggered DC, DEX-treated CD40-triggered DC or immature DC. The proliferative response was measured on day 5.

[0016] Th1 stimulation assays: Hsp65-specific T-cells were cultured with different numbers of HLA-DR matched CD40-triggered DC or with DEX-treated CD40-triggered DC pulsed with the hsp65 protein or with the specific p3-13 peptide epitope. The proliferative response and the T-cell dependent IFN-g production were analyzed on day 3. Data are representative of 4 independent experiments.

[0017] Fig. 6. DEX-treated DC triggered through CD40 induce a state of hyporesponsiveness in Th1 cells. Hsp65-specific T-cells precultured with CD40-triggered DC or with DEX-treated CD40-triggered DC pulsed with the p3-13 peptide epitope were harvested after 48h, allowed to rest in the presence of 5U/ml IL2 for 3 days, and restimulated with p3-13-pulsed DC. The proliferative response and IFN-g production were measured on day 3. Similar results were obtained in 2 independent experiments.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0018] The dendritic cells of the invention possess different capabilities than those previously reported for dendritic cells. One can, therefore, consider these cells to be part of a class of cells distinct from the class formed by the "classical" dendritic cells. The dendritic cells of the invention can be used in a different way than the classical dendritic cells. The dendritic cells of the invention can, for instance, be used to suppress, at least in part, an undesired immune response in a host. In one aspect, the invention, therefore, provides a method for preparing a pharmaceutical composition for reducing an unwanted T-cell response in a host, comprising culturing peripheral blood monocytes from the host to differentiate into dendritic cells, activating said dendritic cells in the presence of a glucocorticoid hormone and loading the activated dendritic cells with an antigen against which the T-cell response is to be reduced. An unwanted T-cell response can be any type of T-cell response. For instance, but not limited to, a T-cell response associated with an autoimmune disease or a transplantation disease, such as a graft versus host disease or a host versus graft disease. A pharmaceutical composition of the invention typically comprises a

dendritic cell of the invention suspended in a liquid suitable for preserving the function of the dendritic cell in the liquid and/or suitable for administration to a host. A host, preferably, is a human. Preferably, the host is at risk of developing or is suffering from an autoimmune disease or allergy. Preferably, the host suffers from or is at risk of suffering from a host versus graft disease and/or a graft versus host disease. With the term "at risk," it is meant that one expects that the host may develop the disease, for instance, but not limited to, a host receiving a transplant. Such a host is considered to be at risk of developing a host versus graft disease. An antigen typically is a peptide capable of binding to a major histocompatibility complex (MHC) I and/or II molecule. Such peptides are known in the art and a person skilled in the art is capable of determining whether a given peptide comprises an antigen or not. An antigen may be derived from a naturally occurring protein. An antigen may also be a synthetic peptide or equivalent thereof, preferably with an amino-acid sequence equivalent to a peptide derived from a protein.

[0019] In another aspect, the invention provides a pharmaceutical composition for reducing an unwanted T-cell response in a host, the composition being obtained by culturing peripheral blood monocytes from the host to differentiate into dendritic cells, activating the dendritic cells in the presence of a glucocorticoid hormone and loading the activated dendritic cells with an antigen against which the T-cell response is to be reduced. In one embodiment, a method is provided for reducing an unwanted T-cell response in a host comprising administering a composition of the invention to the host.

[0020] The invention further provides a method for reducing an unwanted T-cell response in a host comprising culturing peripheral blood monocytes from the host to differentiate into dendritic cells, activating the dendritic cells and/or their precursors in the presence of a glucocorticoid hormone and loading the activated dendritic cells with an antigen against which the T-cell response is to be reduced and administering the composition to the host.

[0021] In one embodiment of the invention, the activation is done through a CD40 receptor. Activation of DC through triggering of the CD40 receptor can involve either incubation with a CD8-CD40L fusion protein, a trimeric form of CD40L consisting of CD40L-molecules to which a modified leucine zipper has been attached, anti-CD40 antibodies, or cells that express CD40L. Other signals that can be employed for the activation of DC as described in the present invention include lipopolysaccharide (LPS) and polyI/C.

[0022] In another aspect, the invention provides a method for obtaining a dendritic cell capable of tolerizing a T-cell for an antigen comprising providing the dendritic cell with a glucocorticoid hormone, activating the dendritic cell and providing the dendritic cell with the antigen. With the term "tolerizing," it meant that the dendritic cell has an immunosuppressive effect on the T-cell. A tolerized T-cell essentially will not respond with cell division when exposed to a cell presenting an antigen, a T-cell in the untolerized state would respond to such exposure with cell division. A tolerized T-cell essentially will not respond by killing a cell presenting an antigen, a T-cell in the untolerized state would respond to such exposure by killing the cell presenting an antigen.

[0023] In one embodiment, the dendritic cell and/or a precursor thereof is provided with a glucocorticoid hormone *in vitro*. A T-cell of the invention is preferably an antigen specific T-cell, a cytotoxic T-cell or a Th cell.

[0024] In another aspect, the invention provides an isolated dendritic cell capable of modifying the function of an antigen specific Th cell, which would otherwise enhance a given immune response, resulting in a T-cell that is capable of reducing this immune response. In one embodiment, the invention provides a method for modifying an antigen specific T-cell comprising providing an dendritic cell according to the invention with said antigen and cocultivating said T-cell and said dendritic cell. Preferably, said cocultivating is performed *in vitro*. The method may further comprise multiplying the functionally modified T-cell.

[0025] The invention also provides an isolated functionally modified T-cell obtainable by a method according to the invention.

[0026] In another aspect, the invention provides the use of a glucocorticoid hormone for obtaining a dendritic cell capable of functionally modifying a T-cell.

[0027] The invention also provides a pharmaceutical composition comprising a dendritic cell and/or a functionally modified T-cell. The invention further provides the use of a dendritic cell and/or a functionally modified T-cell for the preparation of a medicament.

[0028] The invention also provides a method for the treatment of an individual suffering from, or at risk of suffering from, a disease associated with at least part of the immune system of the individual, including providing the individual with a dendritic cell and/or a functionally modified T-cell. Preferably, the dendritic cell and/or the functionally modified T-cell or precursors thereof are derived from an HLA-matched donor. Preferably, the HLA-matched donor is the individual.

[0029] Method of treatments of the invention are preferably used for the treatment of an individual suffering from an autoimmune disease, an allergy, a graft versus host disease and/or a host versus graft disease.

Examples

Example 1

Impairment of CD40-CD40L-mediated phenotypic changes by DEX

[0030] We explored the impact of DEX on the phenotypic changes induced by CD40 ligation on immature monocyte-derived DC. In the absence of DEX, the fusion protein CD8-CD40L induced a strong upregulation of the costimulatory molecules CD80, CD86 and CD40, of the MHC class I and II molecules, of the adhesion markers CD54 and CD58 and of the DC maturation marker CD83 (Fig 1). In the presence of DEX, these CD8-CD40L-induced phenotypic changes were dramatically impaired: the upregulation of CD80, CD86, CD40, CD54, CD58 and of the MHC class I and II molecules was largely inhibited and CD83 was not expressed (Fig 1). Importantly, DEX-treated DC did not revert to a monocyte/macrophage stage as shown by the lack of expression of CD14 (Fig 1). Titration of DEX showed a complete inhibition of CD40-mediated phenotypic changes at 10^{-6} M and 10^{-7} M, a partial blockade at 10^{-8} M and no effect at 10^{-9} M and 10^{-10} (data not shown). In addition, DEX action was dependent on binding to the GC-receptor, since it was asbolished by simultaneous addition of the GC receptor antagonist RU486 (data not shown). In experiments performed with LPS or TNF-a as activation agents, similar results were obtained. However, the combination of DEX and TNF-alpha induced a massive cell death (viable cell recovery 5-10% of control cultures), a phenomenon that was not observed when DEX-treated DC were stimulated with LPS or through CD40 (viable cell recovery 60 to 100% of control cultures) (not shown).

[0031] We next analyzed whether activated DC could still be affected by DEX. DC incubated with CD8-CD40L for 48h and further exposed to DEX maintained a stable activated phenotype (Fig 2).

[0032] We conclude that DEX prevents the phenotypic changes induced by CD40 signals on immature DC and that already activated DC are resistant to DEX action.

Example 2

DEX does not interfere with the regulation of DC antigen uptake machinery

[0033] Unlike activated DC, immature DC efficiently internalize antigens through macropinocytosis and mannose receptor-mediated endocytosis (2, 3, 25, 26). We analyzed whether DEX could affect the DC antigen capture machinery and its downregulation following CD40 cross-linking. As shown in Fig 3, incorporation of FITC-BSA and FITC-mannosylated BSA by immature DC and by DEX-treated immature DC was comparable. Upon CD40 triggering, a similar decrease of FITC-BSA and FITC-mannosylated BSA uptake by both DEX-treated and untreated DC was observed (Fig 3). These results were the first to indicate to us that DEX does not block all aspects of DC activation, since it does not interfere with the down-regulation of the DC antigen capture machinery.

Example 3

DEX-treated CD40-triggered DC secrete IL-10 instead of IL-12

[0034] A key feature of CD40-triggered DC for initiating T-cell immunity resides in their ability to produce the proinflammatory cytokine IL-12 (5, 6, 27). We investigated whether DEX affected IL-12 production by DC stimulated through CD40, and we explored the possibility that DEX could promote the secretion of the anti-inflammatory cytokine IL-10. As shown in Fig 4, CD40 triggering of DC strongly induced IL-12p40 and IL-12p70 secretion (up to 120ng/ml and 170pg/ml, respectively) but only poorly stimulated the production of IL-10 (up to 68pg/ml). In contrast, CD40 triggering of DEX-treated DC resulted in a dramatically reduced IL-12p40 production (up to 100 fold) and in the complete suppression of IL-12p70 secretion, whereas IL-10 production was strongly enhanced (up to 50 fold) (Fig 4). Immature DC and their DEX-treated counterparts failed to secrete detectable amounts of IL-12 and IL-10 (Fig 4). Therefore,

CD40 ligation of DC in the presence of DEX triggers the secretion of high levels of the anti-inflammatory cytokine IL-10 instead of IL-12.

Example 4

DEX-treated CD40-triggered DC are capable of suppressing Th1-type immunity

[0035] The strikingly modified response of DC to CD40 ligation in the presence of DEX prompted us to compare the T-cell stimulatory potential of these cells with that of their DEX-untreated counterparts. In an allogeneic MLR, CD40-triggered DC induced a strong proliferative T-cell response, whereas the addition of DEX prior to CD40 triggering reduced their T-cell stimulatory capacity to that of immature DC (Fig 5). When tested for their ability to stimulate an hsp65-specific $CD4^+$ Th1 clone, CD40-triggered DC pulsed with the hsp65 protein or with the specific peptide epitope p3-13 were found to be potent inducers of both T-cell proliferation and T-cell dependent IFN-g production (Fig 5). In contrast, in the presence of Ag-pulsed DEX-treated CD40-triggered DC, T-cell proliferation and IFN-g production were significantly decreased ($p<0.001$ and $p<0.01$ respectively) (Fig 5). We next investigated whether DEX-treated CD40-triggered DC were simply poor stimulators of Th1 cells or whether they could exert suppressive effects on these T-cells. We, therefore, tested hsp65-specific T-cells stimulated with p3-13-pulsed DEX-treated CD40-triggered DC for their capacity to respond to a second potent antigenic challenge. Fig 6 shows that preculturing T-cells with CD40-triggered DC led to a strong T-cell proliferation and IFN-gamma production upon second antigen-specific restimulation. In contrast, preculture with DEX-treated CD40-triggered DC resulted in a dramatically reduced proliferative and IFN-gamma production capacity of Th1 cells. Thus, CD40 triggering of DC in the presence of DEX results in APC that are not merely poor inducers of T-cell responses but that also induce a state of hyporesponsiveness in Th1 cells.

Materials and Methods

Generation of DC

[0036] Immature DC were generated from peripheral blood monocyte precursors (PBMC). Human PBMC from healthy donors, isolated through Ficoll-Hypaque density centrifugation were plated at $1.5x10^7$ per well in 6-well plates (Costar Corp., Cambridge, MA) in

RPMI 1640 (Life Technologies, Paisley, Scotland) supplemented with 2mM glutamine, 100UI/ml penicillin and 10% FCS. After 2h at 37°C, the nonadherent cells were removed and the adherent cells were cultured in medium containing 500U/ml IL-4 (Pepro Tech Inc. Rocky Hill, NJ) and 800U/ml GM-CSF (kindly provided by Dr S. Osanto, LUMC, Leiden, NL) for a total of 7 days.

Activation of immature DC with a CD8-CD40L fusion protein

[0037] Activation of DC though CD40 was performed with a fusion protein made of the extracellular domain of human CD40L and of the murine CD8a chain (CD8-CD40L). The CD8-CD40L cDNA described by Garrone et al. (23) was transferred into an eukaryotic expression vector containing the hygromycin resistance gene and used for the generation of stably transfected Chinese Hamster Ovary (CHO) cells. Culture supernatants containing the CD8-CD40L fusion protein were concentrated with a pressurized stirred cell system (Amicon, Inc., Beverly, MA), checked for binding to CD40 and tested for optimal DC activation conditions (not shown). DC were incubated at 5×10^5/ml/well in a 24-well plate (Costar Corp., Cambridge, MA) and activated in the presence of 1/10 CD8-CD40L supernatant. Cells and supernatants were analyzed after 48h. Of note, control supernatants obtained from untransfected CHO cells or from CHO cells transfected with the CD8a cDNA lacked DC activating functions and were similar to culture medium.

DEX and RU486 treatment of DC

[0038] Seven days immature DC were treated with 10^{-6}M DEX (Sigma, St Louis, MO) in the presence of GM-CSF and IL-4 or GM-CSF alone. After 24h, DC were analyzed or were further stimulated via CD40 by adding the CD8-CD40L fusion protein to the cultures as described above. In some experiments, the glucocorticoid receptor antagonist RU485 (Roussel-UCLAF, Romainville, France) was used at 10mM final concentration, alone or in combination with DEX.

Analysis of DC surface phenotype by flow cyttometry

[0039] Cells were stained on ice with FITC or PE-conjugated mouse monoclonal antibodies (MoAb) for 30 min in PBS 1% FCS and were analyzed on a FACScan® (Becton Dickinson, San Jose, CA). The following MoAb were used: FITC-anti-CD80 (BB1), PE-anti-

CD86 (FUN-1), FITC-anti-CD40 (5C3), PE-anti-CD54 (HA 58) and PE-anti-CD58 (1C3) (Pharmingen, San Diego, CA); PE-anti-CD14 (L243) and PE-anti-HLA-DR (Mf-P9) (Becton Dickinson); PE-anti-CD83 (HB15A) (Immunotech, Marseille, France); and PE-anti-HLA class I (Tu 149) (Caltag Laboratories, Burlingame, CA).

Antigen uptake experiments

[0040] DC were resuspended in medium buffered with 25mM Hepes. FITC-BSA and FITC-mannosylated BSA (both from Sigma) were added at 1mg/ml final concentration and the cells were incubated at 37°C or at 0°C to determine background uptake. After 1h, DC were washed extensively with ice-cold PBS and analyzed by FACS® using propidium iodide to eliminate dead cells.

Cytokine detection by ELISA

[0041] Culture supernatants were analyzed in serial two-fold dilutions in duplicate. IL-12p70 was detected using a solid phase sandwich ELISA kit (Diaclone Research, Besancon, France) (sensitivity 3pg/ml). For IL-12p40 and IFN-g detection, capture MoAb and polyclonal biotinylated detection Ab were obtained from Peter van de Meijde (BPRC, Rijswijk, NL) (sensitivity 10pg/ml). IL-10 was detected using the Pelikine compact human IL-10 ELISA kit (CLB, Amsterdam, NL) (sensitivity 3pg/ml).

Allogeneic mixed lymphocyte reaction (MLR)

[0042] Nonadherent allogeneic adult PBMC from an unrelated individual were cultured in 96-well flat-bottom plates (Costar Corp., Cambridge, MA) at a density of 1.5×10^5/well with various numbers of g-irradiated (3,000 rads) DC, in triplicate. Proliferation was assessed on day 5 by [^{3}H]thymidine uptake (0.5mCi/well, specific activity 5Ci/mMol, Amersham Life Science, Buckinghamshire, UK) during a 16h pulse.

Th1 stimulation assays

[0043] The Mycobacterium tuberculosis and *M. leprae* hsp65-specific, HLA-DR3-restricted CD4+ Th1 clone Rp15 1-1 used in this study recognizes an hsp65 determinant

corresponding to peptide residues 3 to 13 (p3-13) (24). HLA-DR-matched DEX-treated immature DC and their DEX-untreated counterparts were pulsed with 10mg/ml of p3-13 or with 10mg/ml of hsp65 for 2h, washed extensively and stimulated through CD40 as described above. For Ag-pulsed DEX-treated immature DC, CD40 triggering was performed in the presence of DEX. Hsp65 specific T-cells (10^4) were cultured with different numbers of g-irradiated (3,000 rads) DC in 96-well flat-bottom plates (Costar Corp.) in triplicate for 3 days. [^{3}H]thymidine (incorporation) was measured on day 3 after a 16h pulse. Before the addition of [^{3}H]thymidine, 50ml of supernatants were collected from each well and supernatants from triplicate wells were pooled to measure IFN-g production. To test hsp65-specific T-cells responsiveness to a second potent antigenic challenge, 10^4 T-cells were first cultured for 48h with 5×10^3 peptide-pulsed DC prepared as above then harvested and allowed to rest in medium containing 5U/ml IL-2. Three days later, 10^4 viable T-cells were restimulated with 5×10^3 peptide-pulsed DC generated from the same donor as used for the first culture and tested for their ability to proliferate and to produce IFN-g as previously described.

Statistical analysis

[0044] Covariance analysis was used to compare T-cell proliferation and IFN-g production as a function of DC number, between DEX-treated CD40-triggered DC and DEX-untreated CD40-triggered DC (Fig. 5).

REFERENCES

1. Banchereau J, Steinman RM: Dendritic cells and the control of immunity. Nature 392: 248, 1998.

2. Sallusto F, Lanzavecchia A: Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony stimulating factor and downregulated by tumor necrosis factor a. J Exp Med 179: 1109, 1994.

3. Sallusto F, Cella M, Danieli C, Lanzavecchia A: Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J Exp Med 182: 389, 1995.

4. Caux C, Massacrier C, Vandervliet B, Dubois B, van Kooten C, Durand I, Banchereau J: Activation of human dendritic cells through CD40 cross-linking. J Exp Med 180: 1263, 1994.

5. Cella M, Sheidegger D, Palmer-Lehman K, Lane P, Lanzavecchia A, Alber G: Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T-cell stimulatory capacity: T-T help via APC activation. J Exp Med 184: 747, 1996.

6. Koch F, Stanzl U, Jennevin P, Janke K, Heufler C, Kampgen E, Romani N, Schuler G: High levels IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. J Exp Med 184: 741, 1996.

7. Trinchieri G: Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol 13: 251, 1995.

8. Kamanakà M, Yu P, Yasui T, Koshida K, Kawaba T, Horii T, Kishimoto T, Kikutani H: Protective role of CD40 in Leishmania major infection at two distinct phases of cell-mediated immunity. Immunity 4: 275, 1996.

9. Yang Y, Wilson JM: CD40 ligand-dependent T-cell activation: requirement of B7-CD28 signaling through CD40. Science 273: 1862, 1996.

10. Mackey MF, Gunn JR, Ting P, Kikutani H, Dranoff G, Noelle RJ, Barth Jr RJ: Protective immunity induced by tumor vaccines requires interactions between CD40 and its ligand CD154 Cancer Res 57: 2569, 1997.

11. Grewal IS, Foellmer HG, Grewal KD, Xu J, Hardardottir F, Baron JL, Janeway Jr CA, Flavell RA: Requirement for CD40 ligand in costimulation induction, T-cell activation and experimental allergic encephalomyelitis. Science 273: 1864, 1996.

12. Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, Hong X, Thomas D, Fechner JH, Knechtle SJ: CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. Proc Natl Acad Sci USA 94: 8789, 1997.

13. Durie FH, Aruffo A, Ledbetter J, Crassi KM, Green WR, Fast LD, Noelle RJ: Antibody to the ligand of CD40, gp39, blocks the occurrence of the acute and chronic forms of graft-vs-host disease. J Clin Invest 94: 1333, 1994.

14. Almawi WY, Beyhum HN, Rahme AA, Rieder MJ: Regulation of cytokine and cytokine receptor expression by glucocorticoids. J Leukoc Biol 60: 563, 1996.

15. Moser M, De Smedt T, Sornasse T, Tielemans F, and Chentoufi AA: Glucocorticoids down-regulate dendritic cell function in vitro and in vivo. Eur J Immunol 25: 2818, 1995.

16. Kitajima T, Ariitzumi K, Bergstresser PR, Takashima A: A novel mechanism of glucocorticoid-induced immune suppression: the inhibition of T-cell-mediated terminal maturation of a murine dendritic cell line. J Clin Invest 98: 142, 1996.

17. Viera PL, Kalinski P, Wierenga EA, Kapsenberg ML, de Jong EC: Glucocorticoids inhibit bioactive IL-12p70 production by in vitro-generated human dendritic cells without affecting their T-cell stimulatory potential. J Immunol 161: 5245, 1998.

18. Romani N, Gruner S, Brang D, Kampgen E, Lenz A, Trockenbacher B, Konwalinka G, Fritsch PO, Steinman RM, Schuler G: Proliferating dendritic cell progenitors in human blood. J Exp Med 180: 83, 1994.

19. Randolph GC, Beaulieu S, Lebecque S, Steinman RM, Muller WA: Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. Science 282: 480, 1998.

20. Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu Y-J: Reciprocal control of T helper cell and dendritic cell differentiation. Science 283: 1183, 1999.

21. Bender A, Sapp M, Schuler G, Steinman RM, Bhardwaj N: Improved methods for the generation of dendritic cells from non proliferating progenitors in human blood. J Immunol Methods 196:121, 1996.

22. Romani N, Reider D, Heuer M, Ebner S, Kampgen E, Eibl B, Niederwieser D, Schuler G: Generation of mature dendritic cells from human blood: an improved method with special regard to clinical applicability. J Immunol Methods 196:137, 1996.

23. Garrone P, Neidhardt E-M, Garcia E, Galibert L, van Kooten C, Banchereau J: Fas ligation induces apoptosis of CD40-activated human B lymphocytes. J Exp Med 182: 1265, 1995.

24. Geluk A, van Meigaarden KE, Janson AAM, Drijfhout J-W, Meloen R, de Vries RRP, Ottenhoff T: Functional analysis of DR17(DR3)-restricted mycobacterial T-cell epitopes reveals DR17 binding motif and enables the design of allele-specific competitor peptides. J Immunol 149: 2864, 1992.

25. Engering AJ, Cella M, Fluitsma D, Brockhaus M, Hoefsmit ECM, Lanzavecchia A, Pieters J: The mannose receptor functions as a high capacity and broad specificity antigen receptor in human dendritic cells. Eur J Immunol 27: 2412, 1997.

26. Tan MCAA, Mommaas AA, Drijfhout J-W, Jordens R, Onderwater JJM, Verwoerd D, Mulder AA, van der Heiden AN, Scheidegger D, Oomens LCJM, Ottenhoff THM, Tulp A, Neefjes JJ, Koning F: Mannose receptor-mediated uptake of antigens strongly enhances HLA class II-restricted antigen presentation by cultured dendritic cells. Eur J Immunol 27: 2426, 1997.

27. de Saint Vis B, Fugier-Vivier I, Massacrier C, Gaillard C, Vanbervliet B, Ait-Yahia S, Banchereau J, Liu Y-J, Lebecque S, Caux C: The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. J Immunol 160: 1666, 1998.

28. Piemonti L, Monti P, Allavena P, Sironi M, Soldini L, Leone BE, Socci C, Di Carlo V: Glucocorticoids affect human dendritic cell differentiation and maturation. J Immunol 162: 6473, 1999.

29. Blotta MH, Dekruyff RH, Umetsu DT: Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes. J Immunol 158: 5589, 1997.

30. Visser J, van Boxel-Dezaire A, Methrost D, Brunt T, de Kloet ER, Nagelkerken L: Differential regulation of interleukin 10 (IL-10) and IL-12 by glucocorticoids in vitro. Blood. 91: 4255, 1998.

31. van Kooten C, Banchereau J: Function of CD40 on B cells, dendritic cells and other cells. Curr Opin Immunol 9: 330, 1997.

32. Scheinman RI, Cogswell PC, Lofquist AK, and Baldwin Jr AS: Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. Science 270: 283, 1995.

33. Auphan N, Dinato JA, Rosette C, Helmberg A, Karin N: Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. Science 270: 286, 1995.

34. Murphy TL, Cleveland MG, Kulesza P, Magram J, Murphy KM: Regulation of interleukin 12 p40 expression through an NF-kappa B half site. Mol Cell Biol 15: 5258, 1995.

35. Yoshimoto T, Nagase H, Ishida T, Inoue J, Nariuchi H: Induction of interleukin-12 p40 transcript by CD40 ligation via activation of nuclear factor-kappaB. Eur J Immunol 27: 3461, 1997.

36. Ridge JP, Di Rosa F, Matzinger P: A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. Nature 393: 474, 1998.

37. Bennett SR, Carbone FR, Karamalis F, Flavell RA, Miller JF, Heath WR: Help for cytotoxic-T-cell responses is mediated by CD40 signalling. Nature 393: 478, 1998.

38. Schoenberger SP, Toes REM, van der Voort EI, Offringa R, Melief CJM: T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature 393: 480, 1998.

ABSTRACT OF THE DISCLOSURE

The present invention provides novel methods for immunotherapy. The invention provides immune cells and methods to generate them, with the capacity to at least in part reduce an immune response in a host. In one aspect, the invention provides a method for generating a dendritic cell with the capacity to tolerize a T-cell for antigen the T-cell was specific for including culturing peripheral blood monocytes from an individual to differentiate into dendritic cells, activating the dendritic cells in the presence of a glucocorticoid hormone and loading the activated dendritic cell with the antigen the T-cell was specific for.

2183\4205.1\Patent Application.doc 10/17/01